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BACTERIAL WILT OF SOY[illegible]

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ON BACTERIAL WILT OF SOYBEANS IN THE UKRAINE

/Following is the translation of an article by V. A. Muras in the Ukrainian-language periodical Mikrobiolohichnyy Zhurnal (Microbiology Journal), Vol 25, No 5, 1963, pages 42-49./

The existing findings in the literature by native and foreign authors indicate that the soybean is damaged by twelve species of inciters of bacterial diseases representing several genera.

The most extensive and injurious bacterial diseases are caused by Pseudomonas glycinea Coeiper (and its varieties) and Xanthomonas phaseoli var. sojense Hedges.

The bacteria mentioned cause spottiness on the soybean leaf. A considerable body of research is devoted to the study of these bacterioses and their causative agents. Only isolated works are known on bacterial wilt of soybeans.

Thus G. K. Burgvits /_/ reports a disease of the soybean which appears in the injured vascular system of the plant and causes dwarfism, sudden wilting, and leaf wrinkle. The causative agent of this disease is the polyphagous bacteria Ps. solanacearum Smith.

A. P. Klykov /6/ in a work on cotyledonous bacteriosis of soybeans mentions that two species of bacteria were systematically encountered when inoculating seed and diseased cotyledons. One of them, Ps. solanacearum Smith, was more frequently inoculated. His article says nothing about wilt of the adult soybean plants.

The conclusion may be drawn from the findings of that author that Ps. solanacearum is easily isolated from the diseased plants and is therefore often found in soybeans.

We must take into consideration that V. I. Vzorov [4], who studied more than 1700 pure cultures and gave a table of the composition of bacterial causative agents in the USSR according to species, does not mention Ps. solanacearum on soybeans.

A report by Van der Wolk (quoted by Yachevskiy [7]) appeared in the foreign literature on this question as early as 1910 and disclosed bacterial wilt in soybeans and peanuts in Java.

In 1919 Smith and McCulloch [9] published a short article on Ps. solanacearum damage to beans and other legumes, among which they note soybeans. The authors indicate the ... of the disease as does G. K. Burgvits. The investigators emphasize that plants are especially susceptible to this bacterium in the early stage of development.

The findings of F. A. Wolf [...] are that there is less bacterial wilt in soybeans than in other plants because of the considerable woodiness of the stems of the soybean.

Soybean wilt may also be fusarial in nature [..., 5, etc.]. The leaves of these sick plants droop, curl ..., get ..., and then drop off. The stem tissues at the collum become brown and in damp weather a mycelial fur of pinkish or orange color appears on them.

We studied bacterial diseases of soybeans in the Ukraine in 1956-1961.

The present article gives only the results of the study of bacterial soybean wilt detected by us in Ukrainian territory.

When investigating the soybean samples collected from different points in the Ukrainian SSR (field of the Chernivets'kyy and Kyrovohrads'kyy Experimental Stations; at the Experimental Base of the Institute of Microbiology of the Academy of Sciences, Ukrainian SSR, in Feofaniya; one strain obtained from samples from the Kuban) we isolated the strains of the causative agents of bacterial soybean disease which under definite conditions and in definite phases of the plants' growth caused the plants to wilt.

The bacteria were inoculated from different parts of the soybean plants--from the leaves, the dark-brown streaks near the ribs of the leaves, the brown streaks on the stems, the ... fissures on the stems, and the brown spots on the pods which went over onto the seeds in the form of light brown spots.

The leaf lesions had brown and greenish-brown oblong amorphous spots slightly translucent in the light; some of them were surrounded with a weak yellow aureole. From the lower surface of the leaf blade the spots had the same appearance, but were a little lighter. The

tissue in the affected parts often dropped out. At times the leaves gave signs of wilting, and the affected spots were darker in color, as though oiled.

According to our data the above-noted type of disease is of slight extent in the Ukraine, being found in isolated cases. This is indicated by the mere nine strains of bacteria of this species isolated from upwards of 1206 specimens of diseased soybean plants.

A study of the cultural, morphologico-biochemical, pathogenic, and serological properties of this group of causative agents made it possible to characterize them.

They are mobile rods 1.1-1.5 x 0.4-0.6 microns in size with 1-3 flagella, Gram-negative, aerobic, with greyish-white colonies on potato agar, rounded, glistening, flat, transparent, with solid and slightly raised centers. The colonies have undulant edges.

Table 1 gives the principal characteristics of the agents examined.

We studied the pathogenic properties of these bacteria in soybeans in different periods of growth, as well as in other plants. Infection was accomplished by a day-old culture of microorganisms which contained 1,000,000,000 bacteria cells per milliliter.

The strains gave the typical picture of wilt on the second day in soybeans grown in flowerpots (Fig. 1). In the cotyledons deep depressions appeared at the points of injection with 3 x 4 mm dark-brown spots.

In all the experiments the control was injected with sterile water instead of the agent.

Soybeans growing in flats displayed the same picture when infected, but in a rather milder form. There were dark-brown depressed spots on the cotyledons, even going through to the other (upper) side.

Darkening of the leaf ribs at the injection points (the "network") occurred when the leaf blades were infected, and the leaves progressively dried up (Fig. 2). The result of the injection into the rib the leaf lost its <u>turgor vitalis</u>, drooped, and wilted, all in 2 to 4 days after inoculation.

When adult soybean plants were infected at the Experimental Base of the Institute of Microbiology of the Academy of Sciences, Ukrainian SSR, at Feofaniya not all plants were observed to wilt. The plants were infected when young and when their beans were ripe.

Table 1. Principal Characteristics of Causative Agent of Wilt-Type Bacterioses

Ps. solanacearum f sojense	
Mobility	+
Gram reaction	-
Aerobe	+
Gelatine attenuation	+
Nitrate reaction	-
Color of colonies	greyish-white
Starch hydrolysis	-
Glucose	
acid	+
gas	-
Lactose	
acid	-
gas	-
Sucrose	
acid	+
gas	-
Maltose	
acid	-
gas	-
Mantite	
acid	+
gas	-
Raffinose	
acid	+
gas	-
Xylose	
acid	+
gas	-
Rhamnose	
acid	+
gas	-
Salicin	
acid	+
gas	-
Arabinose	
acid	+
gas	-
Dextrin	
acid	-
gas	-
Fructose	
acid	+
gas	-
Dulcite	
acid	+
gas	-
Tartaric acid	
acid	-
gas	-

Ps. solanacearum f sojense	
Succinic acid	
acid	alkali
gas	
Citric acid	
acid	alkali
gas	
Glycerin	
acid	+
gas	-
Cellulose	
acid	-
gas	-
Cellulose--chalk	
acid	-
gas	-
Salicylic acid	
acid	-
gas	-
Acetic acid	
acid	-
gas	-
Formic acid	
acid	alkali
gas	
Malic acid	
acid	alkali
gas	
Benzoic acid	
acid	-
gas	-
Galactose	
acid	+
gas	-
Inulin	
acid	-
gas	-
Litmus serum	alkali
Size in microns	
length	1.1-1.5
breadth	0.4-0.6
Spores	-
Capsules	+
Milk	
coagulum	-
peptone	+
Indole	-
Hydrogen sulfide	-
Ammonia	+

GRAPHIC NOT REPRODUCIBLE

Fig. 1. Artificial Infection of Soybeans (Injection into Stem).

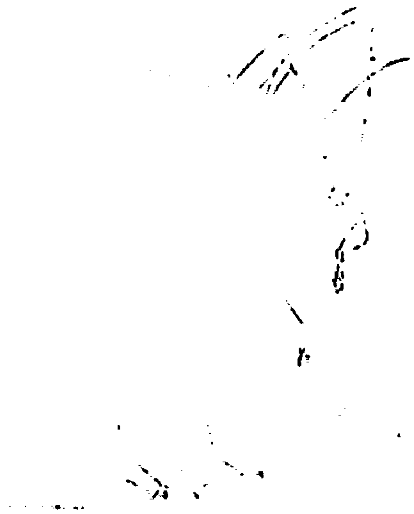

Fig. 2. Withering of Soybean Leaves from Artificial Infection.

GRAPHIC NOT REPRODUCIBLE

After inoculation the leaf blade wrinkled up, and brown spots (4 x 7 mm) with weak chlorosis formed around the points of injection. The infection did not manifest itself so sharply on the leaves of the lower layer, and chlorosis was either entirely absent or hardly perceptible. The spots were lighter at the center, and in time the leaf tissue fell out at these spots, but the plants themselves did not wilt. Stem innoculation did not cause wilting; leaf-stalk inoculation resulted in the appearance of dark-brown, almost black, dry spots 4-5 x 6-8 mm in size. In some cases the spots enlarged, went deep into the tissue, and the leaf-stalk bent down and broke off.

On the third or fourth day after infection dark-brown spots developed on the pods and blackening was noticeable under the thin skin of the pod.

We also studied the pathogenicity of these strains on a number of other plants in order to obtain a more accurate picture of their adaptational characteristics.

In selecting the plants we were guided by the fact that the nightshade family are host plants to Ps. solanacearum, and we chose Fabaceae because they belong to the same family as the soybean.

The following plants were artificially infected in leaf and stem at Feofaniya: tomatoes ("Mayak" and "Marglob" varieties), tobacco (Ternivs'kyy and Havana varieties), potatoes, peas ("Uladivs'kyy 208," "Komsomol," and "Viktoriya Mandorfs'ka" varieties), beans ("Bomaba," "Dryha P'yatyrichka," and "Sparzheva" varieties), white lupine, blue lupine, chick peas, and lentils.

Interesting results were obtained on the two varieties of tomatoes In the early spring infection of the leaves and stems of tomatoes growing in pots indoors gave negative results.

Tomatoes were again inoculated in summer of the same year in Feofaniya. On the third day there formed on the leaves large dry spots with weak concentricity and yellow aureale (4-5 mm). The infection manifested itself better on the leaves of the lower layers. The leaves of the upper layer were without chlorosis; their spots were light-brown and smaller (3 x 4 mm).

In the green fruit blackening of the tissue at the injection points was observed and the affected tissue was dry. When the tomatoes ripened the spots had become field-grey, dry, with dark-grey veins like those caused by Xanth. vesicatoria, but no epidermal cracking was detected. When these tomatoes were cut in two across the spots hardening of the tissue from the spot to a depth of 4-5 mm was apparent, the rest of the tissue remaining healthy.

Dark spots (7 x 3 mm) appeared on the stems at the points of injection and the plant did not wilt.

The tomatoes were once more inoculated in August when the plants were already old. Infection in the stem and leaves gave negative results A different picture was observed in the fruit. The red fruit began to rot. Oily dark-hued spots 4-5 cm in diameter form, the tissue became soft, and decay developed. Then other microorganisms and fungi established themselves there.

In the control (injected with sterile water) the spots were white-grey, dry, and 2 x 4 mm in size.

In the tobacco brown spots 6-10 mm long were observed only on the leaf ribs, on the stems they were 3-4 mm long.

When a vessel became black (injection in the rib) the tissue broke through in the center and contracted. In one case (Ternivs'kyy variety) the darkening went 3.5 cm further along the vessel. No further spread or development of the disease was observed. The leaf tissue at the injection points became dark.

The culture proved to be nonpathogenic to potatoes.

A number of Fabaceae plants were also artificially infected with the strains isolated.

The bacteria proved to be pathogenic for the "Viktoriya Mandorfs'ka" variety of peas, the "Bomba" and "Sparzheva" varieties of beans, white and blue lupine, and the horse beans. Tissue necrosis (1.5-4 x 2.5-6 mm) around the injection points, with or without chlorosis, was detected in all these plants. The ribs and stems became brown. In some cases the leaves drooped, but the disease developed no further.

Inoculation gave negative results in the "Udalivsk'kyy 208" and "Komsomol" varieties of peas, the "Praha P'yaty'itka" variety of beans, the chick peas, and the lentils. Artificial infection of lilac also proved unsuccessful.

After having studied the biology of the group of microorganisms in question we assigned the group to Pseudomonas and designated it as a species which because of a number of characteristics is close to Ps. glamaceanus. Gaidl.

Because of its cultural, morphologic-biochemical features this species of bacteria is close to Ps. glycinea, the inciting agent of a very widespread soybean bacteriosis, angular leaf spot.

Our findings show that they differ from each other in that Ps. glycinea does not attenuate gelatine, give alkali in tartaric acid,

nor act on milk. The inciting agents in question attenuate gelatine, leave tartaric acid unchanged, and peptonize milk. The chief difference, however, consists in the picture exhibited by the disease caused by these two microorganisms.

They also differ serologically. Cross agglutination and Castellani's saturation test indicate that they are serologically two different species, although they have common, but not identical, antigens.

The bacteria which we investigated also differ from Ps. syringae and Ps. tabacum. They differ from Ps. solanacearum described by a number of authors [2, 7, 8] in that they attenuate gelatine and give no nitrate reduction.

According to A. A. Yachevskiy [7] and Ch. Elliott [8], moreover, Ps. solanacearum forms a brown pigment. Our cultures did not form it.

Welles and Roldan [10] noted that Ps. solanacearum extracted from chrysanthemums never gave a strong brown pigment; only through subinoculation via tomatoes was the pigment produced.

We attribute this disagreement to the great variability in the characteristics of Ps. solanacearum noted by the authors who investigated the inciter of bacterial wilt.

The group of microorganisms isolated may therefore be regarded as a form of Ps. solanacearum Smith which has adapted itself to parasitism on the soybean. This bacterium is a denizen of the southern latitudes where the course of the disease is rapid and there are distinctly pronounced symptoms of wilt, while it is possible that in the Ukraine the bacteria are just now beginning to adapt themselves to parasitism on plants, since the wilt manifests itself mildly and is found considerably less often.

It is too bad that A. P. Klykov [6] does not in his article describe the microorganisms isolated but only names them because there is some doubt that that investigator was dealing with Ps. solanacearum Smith.

Table 2 lists the comparative characteristics of some of the bacteria of genus Pseudomonas that parasitize the soybean.

Table 2. Comparative Characteristics of Several Bacteria of Genus Pseudomonas Which Parasitize Soybeans

1) Збудник	2) Реакція за Грамом	3) Аероб	4) Розрідження желатини	5) Редукція нітратів	6) Колір колоній	7) Гідроліз крохмалю	8) Глюкоза		9) Лактоза		10) Сахароза		11) Гліцерин		12) Мальтоза		13) Ксилоза		14) Саліцин		15) Маніт		16) Молоко		17) Розмір у мікронах		18) Капсули	19) Індол	20) Сірководень	21) Аміак	22) Рухливість
							23) к-та	24) газ	23) к-та	24) газ	23) к-та	24) газ	23) к-та	24) газ	23) к-та	24) газ	23) к-та	24) газ	25) к-та	24) газ	25) к-та	24) газ	30) зсідання	26) пептон.	27) довжина	28) ширина					
Ps. solanacearum 29)(за Ячевським)	–	+	–	+	34) Білі, поступово темніють	+			–	–	–				–	–							–	+	1,5	0,5	–	–		+	+
Ps. solanacearum 30)(за Elliott Ch.)	–	+		+	35) Опалесціюючі з жовтим пігментом	( )	(·)		(+)	(–)	(+)		+)	(–)	(+)	–)	)		)(	)(–)			–	+	1,5	0,5	–	–	(+)	(+	+
Ps. solanacearum f. *sojense*					36) Сіруваті, ...	–	+	–	–	–	+		+	–	–	–	+	–	–	–	+		–	+	1,1—1,5	0,4—0,6	+	–		+	+
Ps. glycinea 31)(за Coerper F.)	–			–	37) Білі з коричн. пігментом	–	+	–	+		+		+		–	–					+	–	+	–	2,3—3	1,2—1,5	+(на кров. агарі) 40)	+			+
Ps. glycinea 32)(наші штами)	–	+			36) Сіруваті, опалесціюючі	–	+	–	–	–	+	–	+	–	–	–	+	–	–	–	+		–	–	1,4—1,8	0,4—0,5	+	–	–	+	+
Ps. sojae 33)(за Wolf'y)	–	+			38) Білі без пігменту		–	–	–	–	–	–	–	–											1,2—2,6	0,8—0,9	+	–			+
Ps. glycinea var. *japonica* 30)(за Elliott Ch.)	–	+	–	–	38) Білі без пігментів		+	–			+	–													1,6—3,0	0,6—0,8	+	–			+

39) Примітка: У дужках—дані Лаусона (за Еліотт).

[Note: See legend on next page.]

Table 2. Legend: (1) Inciting agent; (2) Gram reaction; (3) Aerobe; (4) Gelatine attenuation; (5) Reduction of nitrates; (6) Color of colonies; (7) Hydrolysis of starch; (8) Glucose; (9) Lactose; (10) Sucrose; (11) Glycerine; (12) Maltose; (13) Xylose; (14) Salicin; (15) Manite; (16) Milk; (17) Size in microns; (18) Capsules; (19) Indole; (20) Hydrogen sulfide; (21) Ammonia; (22) Mobility; (23) Acid; (24) Gas; (25) Coagulum; (26) Pepton; (27) Length; (28) Breadth; (29) (After Yachevskiy); (30) (After Ch. Elliott); (31) (After F. Coerper); (32) (Our strains); (33) (After Wolf); (34) White, gradually darkening; (35) Opalescent with brown pigment; (36) Greyish-white, opalescent; (37) White with brown pigment; (38) White without pigment; (39) Note: In parentheses are Dowson's data (after Elliott); (40) On blood agar.

Conclusions

1. A bacterial wilt-type disease in a somewhat weakened form is encountered in soybean plantings on Ukrainian territory.

2. The spread of this type of disease in the Ukraine in 1959-1961 was insignificant, only isolated cases being observed. The insufficient adaptation of the inciting agent to parasitism under ~~our~~ conditions may explain this.

3. We consider the inciting agent of the mentioned bacteriosis to be a form of Ps. solanacearum Smith which has adapted itself to parasitism on the soybean.

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